Fractionation of the Color of Pure Maple and Other Sirups by Gel Filtration

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By E. E. STINSON and C. O. WILLITS (Eastern Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture, Philadelphia, Pa. 19118)

The colorants of pure maple, cane and maple, refined cane sugar, and light brown sugar sirups were separated into two fractions, one of high- and the other of low-molecular weights, by means of gel filtration.

The ratio of the amounts of high- to the low-molecular weight fractions of pure maple was the lowest of the four sirups and serves as a means of differentiation from these sirups. The color fraction ratio was highest for blended cane-maple sugar sirup.

Many maple sirups are also distinguished by a pink band formed on the gel filtration column.

The formation of maple sirup color during the manufacturing process is of considerable importance because the grade and price of maple sirup is determined by color, with a considerable price differential in favor of the light colored grades. Studies on the formation of color in maple sirup have been underway at this laboratory with the objective of modifying the manufacturing processes in order to minimize the color development.

Gel filtration was used in earlier work by Stinson and Willits (1) to isolate the colorants in a medium amber maple sirup after chloroform extraction of the flavor components. By this procedure the color from this maple sirup was separated into two distinct fractions, one containing colored molecules of high-molecular weight and the other of low-molecular weight.

Based upon this, the present study was made to determine whether the high- and low-molecular weight fractions of maple sirup coloration occur in a fixed ratio in all of the grades of maple sirup and whether they exist in other sugars and sugar sirups in the same ratios.

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Experimental

Sirups

The pure maple sirups representing the four commercial grades were obtained directly from the producers. Most were produced during the 1964 season, although several had been stored at average room temperature for one or more years. The high-flavored maple sirup produced by the method developed in this laboratory (2) had a 5-fold enhancement of flavor and was classed as dark amber on the maple sirup color comparator (3). The sirup blends containing 15% maple sirup were obtained from retail stores. All were rated as medium amber. Only one label specified the addition of artificial colorant (caramel), but such mention is not required in the state where marketed.

The refined and light brown cane sugars were also obtained from local stores. The solid sugars were dissolved in sufficient water to form 65.5° Brix sirups, and the colors were graded according to a maple color comparator scale. The sirup containing refined cane sugar was rated as light fancy, while the sirup from light brown sugar was medium amber.

Separation of Colorant Fractions

Gel filtration has been shown by Stinson and Willits (1) to be effective in separating maple sirup colorants on the basis of size. Particles of a dextran derivative having pores of definite dimensions were used for gel filtration. Molecules having larger dimensions cannot diffuse into the liquid within the pores and consequently are concentrated in the interparticular liquid, while smaller molecules are uniformly distributed both in

the intra- and interparticular liquids. When a concentrated solution containing a mixture of large and small molecules is applied to the top of a column packed with this dextran derivative and the molecules are washed through with water, the large molecules emerge first as they need only to diffuse through the small volume of interparticular liquid; the small molecules emerge later as they must diffuse through a much larger volume of liquid. Fractionation of the eluate emerging from the column permits separation of particles on the basis of size. In addition to this effect, there is evidence that certain compounds are weakly adsorbed on the dextran derivative (4). The sorptive effect is weak, since continued washing with water is sufficient to remove the material from the column.

Glass columns 4 cm i.d. × 85 cm were packed with an aqueous slurry of 295 g of Sephadex G-25, medium grade, from which the fines had been eliminated by repeated washing with water. The excess liquid was removed after the slurry had settled by draining liquid from the bottom of the column at 4 ml per minute until the liquid level was at the top of the Sephadex.

Samples of sirup (65° Brix) diluted from 67 to 100 ml, in order to reduce the viscosity, were applied to the top of the column and washed through the column at 4 ml per minute by water under 4 ft of pressure. The eluate emerging at the bottom of the column was collected in 20 ml fractions. Color was estimated by visual comparison of the fractions with MacAdam color standards (5).

The fractions were also tested for the presence of carbohydrate by the anthrone test (6). The fraction in which sucrose was first detected by the anthrone test indicated the division point between high- and low-molecular weight coloration. The high-molecular weight coloration was contained in the fractions preceding this, while the low-molecular weight coloration was contained in this and subsequent colored fractions. As the concentration of sucrose increased sharply in the fractions following its first detection, it was possible to determine sucrose by refractometer in these fractions. A typical run is plotted in Fig. 1. The fractions containing the respective types of coloration were combined, and the two resulting solutions were each concentrated to 67 ml (the original volume) under a mild vacuum by using a rotary evaporator with the flask immersed in a 30°C water bath. The relative intensities of the high- and low-molecular weight colorants present in each sirup were then measured by a Duboscq color comparator. The results are given in Table 1 and Fig. 2. The pink component indicated in Table 1 usually emerged, upon continued water washings, between fractions 140 and 190. The amount of pink component was too slight to be measured when the fractions were combined and concentrated.

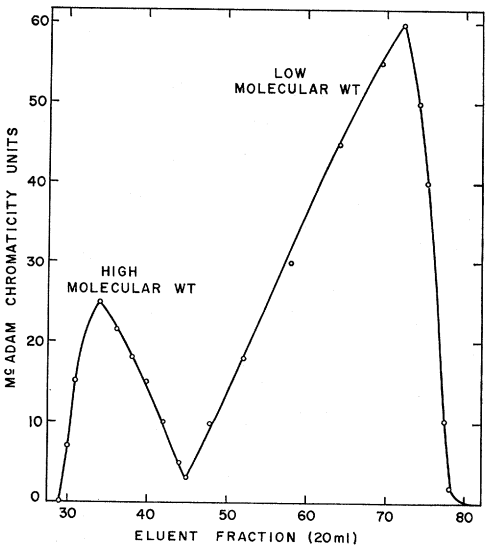

***Fig. 1*—Color in high- and low-molecular weight fractions separated by gel filtration from maple sirup, measured by comparison with MacAdam color standards.**

The infrared spectrum was obtained from a highly purified sample of the high-molecular weight colorants of a medium amber maple sirup. This material had been repurified by concentrating the solution containing high-molecular weight colorant to 1 ml and subjecting it to two successive passes through a gel filtration column 2 × 50 cm, prepared as before. On neither pass was a colored band found in the low molecular weight region. The final weight of the high molecular weight colorant was 0.031 g. The IR

Table 1. Relative amounts of high- and low-molecular weight colorants in maple and other sirups

Sirup Samples	% High Mol. Wt	% Low Mol. Wt	Ratio High:Low Mol. Wt Colorant	Pink Component
Pure Maple				
Fancy				
1. (1963)	25.2	74.8	0.337:1	strong
2. (1964)	21.7	78.3	0.277:1	absent
3. (1964)	25.1	75.9	0.318:1	strong
Light Amber				
4. (1963)	28.2	71.2	0.405:1	strong
5. (1964)	35.7	64.3	0.555:1	weak
6. (1964)	14.0	86.0	0:162:1	absent
Medium Amber				
7. (1964)	13.0	87.0	0.150:1	strong
8. (1964)	6.1	93.9	0.0645:1	strong
9. (1964)	12.9	87.1	0.148:1	weak
Dark Amber				
10. (1961)	35.3	64.7	0.546:1	absent
11. (1964)	21.9	78.1	0.290:1	absent
12. (1964)	20.0	80.0	0.250:1	weak
High Flavored				
13. "5x"	25.2	74.8	0.338	absent
Blended Cane-Maple Sirups				
14. Commercial	75.2	24.8	3.06:1	absent
15. Commercial	45.1	54.9	0.822:1	absent
16. Commercial	51.9	48.1	1.08:1	absent
17. Commercial	65.4	34.6	1.89:1	absent
Cane Sugar				
18. Refined	38.5	61.5	0.625:1	absent
19. Light brown	37.2	62.8	0.592:1	absent

spectrum was determined on a Perkin-Elmer 21 Spectrometer from a water-deposited film on a KRS-5 microplateau (7).

Results and Discussion

Gel filtration completely separated high- and low-molecular weight colorants, as shown by the example given in Fig. 1. Both the high- and low-molecular weight colorants were found in well defined peaks separated by an area of low intensity.

The low-molecular weight colorant showed less homogeneity on the gel filtration columns than the fractions of higher molecular weight, as indicated by the width and tailing of the band. Several sirups had two peaks trailing sucrose in the low molecular weight zone.

The ratio of the amounts of high- to low-molecular weight colorants for pure maple sirups (Table 1 and Fig. 2) ranged from 0.066:1 to 0.546:1, which is lower than the ratios of either the cane sugar sirups (0.59:1–0.63:1) or the commercial cane and maple sirup blends, which ranged between 0.83:1–3.06:1.

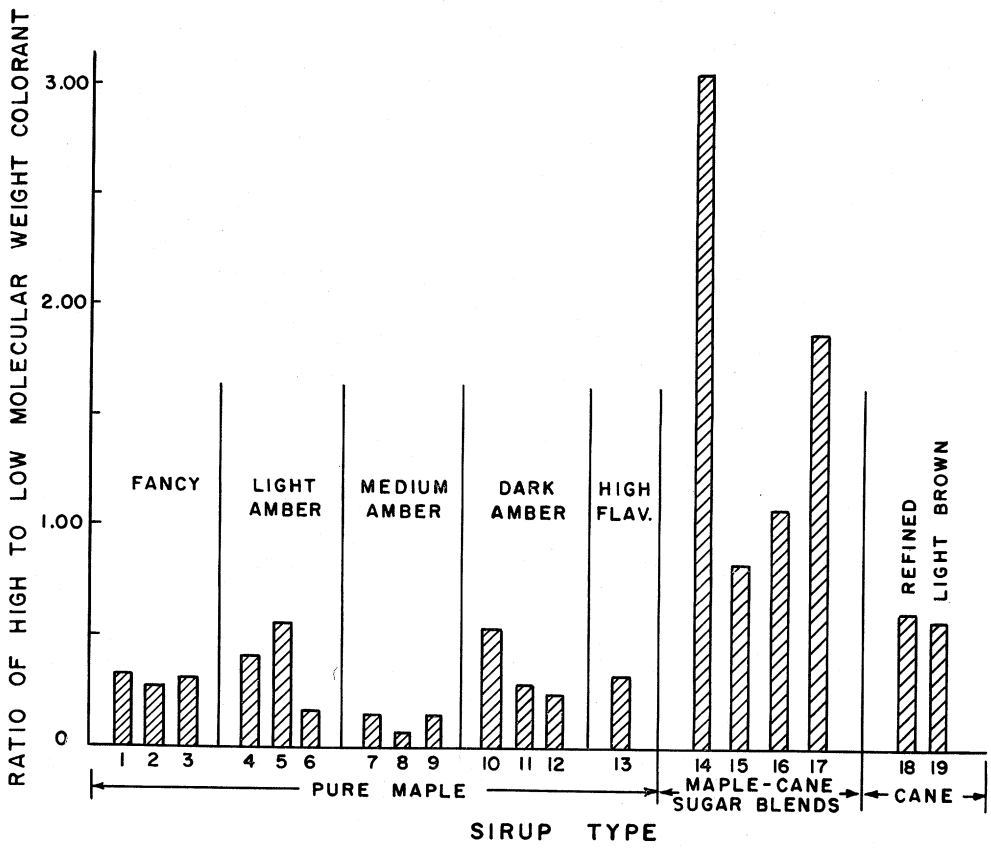

***Fig. 2*—The ratios of high- to low-molecular weight colorants separated from sirups of different types by gel filtration.**

The ratios of high- and low-molecular weight colorants found in both grades of cane sugar (refined and light brown) were higher than the highest value found for pure maple sirup, but it is possible that more extensive sampling would disclose some maple sirups with slightly higher ratios. On the other hand, the ratio values for the commercial blends containing 15% maple sirup were much higher, ranging from 0.822:1 to 3.02:1 or a minimum increase of at least 51% for the lowest commercial blend over the highest pure maple sirup. The low ratio of the amounts of the two color fractions of maple sirup provides a means of differentiating it from the other sirups.

The grade of the pure maple sirup and the age of the samples tested had no discernible effect on the ratio of high- to low-molecular weight colorants.

A pink band indicated in Table 1 was separated on the dextran column in varying intensity in 8 of the 12 pure maple sirups. This component has not hitherto been described in maple sirup. Its slow rate of movement on the column indicated that the material had a slight tendency to be adsorbed on the surface of the dextran. The attractive forces were sufficiently weak so that with continued rinsing with water the pink material could be removed from the column. Although the bands on the column were often prominent, no color was visible in those fractions eluted from the column which should have contained the pink material. Concentration of these fractions resulted only in a brown solution. The pink coloration probably makes little significant contribution to the total coloration of the original sirups. There was no correlation be-

tween the occurrence of the pink colorant and the age or grade of the sirup. No pink band was obtained from high-flavored maple sirup, the commercial blends of cane and maple sirup, nor in the cane sugar sirups.

The infrared spectra of the purified larger, macro, colored molecules showed the same characteristics as the infrared spectrum of a maple colorant fraction previously isolated by Willits, *et al.*, by ion exchange adsorption and dialysis (8). These had adsorption bands typical of carbohydrates, with no bands characteristic of olefinic or carbonyl unsaturation, aromaticity, or carboxyl groups. The low degree of unsaturation and the intense coloration of the isolated pigment suggest that the unsaturated chromophoric centers must have strong tinting properties.

Acknowledgments

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